Chapter 17
Role of Phospholipase C in the α_1-Adrenoceptor Mediated Cardiac Hypertrophy

Paramjit S. Tappia, Adriana Adameova and Naranjan S. Dhalla

Abstract Phospholipase C (PLC) is considered to mediate the cardiomyocyte hypertrophic response to norepinephrine (NE) through activation of α_1-adrenoceptor (α_1-AR). In this review, the role of PLC isozymes in cardiac hypertrophy is highlighted and some of the mechanisms that are involved in the regulation of PLC isozyme gene expression, protein abundance, and activities are identified. The discussion is focussed to highlight the role of PLC in different experimental models of cardiac hypertrophy, transgenic mice, as well as isolated adult and neonatal cardiomyocytes with particular emphasis on α_1-AR-PLC-mediated hypertrophic signals. On the basis of the information available in the literature, it is suggested that molecular modulation of specific PLC isozymes is involved in the α_1-AR mediated response for the initiation and progression of cardiac hypertrophy. Furthermore, different molecular sites in the NE-induced signal transduction pathway are identified to serve as viable targets for the modification of this adaptive mechanism for maintaining cardiac function.

Keywords Phospholipase C · α_1-adrenoceptor · Signal transduction · Cardiac hypertrophy · Pressure overload · Volume overload · Transgenic mice · Neonatal cardiomyocytes Adult cardiomyocytes Phospholipase D

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17.1 Introduction

Although the heart is known to adapt to increased work and hemodynamic load by increasing muscle mass as well as changing the size and shape of the heart, such a remodeling of the myocardium is compensatory at initial stages, but results in cardiac failure at late stages of the development [1, 2]. A moderate increase in the level of hypertrophic hormones including norepinephrine (NE) produces beneficial effects during early stages of cardiac hypertrophy, but prolonged exposure of the hearts to an excessive amount of NE produces deleterious actions at late stages of cardiac hypertrophy [1, 2]. A large body of evidence has revealed that various subcellular organelles including sarcolemma (SL), undergo varying degrees of changes in their biochemical composition and molecular structure in the development of cardiac hypertrophy as well as transition of cardiac hypertrophy to heart failure. This subcellular remodeling occurs due to alterations in cardiac gene expression as well as activation of different signaling proteins including phospholipases. The activation of phospholipase C (PLC) has a number of immediate consequences for signal transduction events in cardiomyocytes, and thus has an integral role to play in subcellular and cardiac remodeling (Fig. 17.1). Under physiological conditions, adrenergic responses are mediated predominantly by the β_1-AR to increase cardiac contractile activity and to influence hypertrophic growth in the long term [3]; however, under pathological conditions signal transduction mechanisms via the α-AR become more apparent and influential in the initiation and progression of cardiac hypertrophy [4]. Diminishing or reversing subcellular remodeling is now emerging as an important therapeutic goal in the treatment or prevention of cardiac hypertrophy and subsequent transition to heart failure in high-risk patients. Accordingly, it is our contention that pharmacological or molecular modulation of the different components of the α_1-AR-PLC signaling axis may represent a viable target.

17.2 The Myocardial α_1-Adrenoceptor Subtypes

The α-ARs are classified into two subtypes; $\alpha_{1A,B,D}$ and $\alpha_{2A/D,B,C}$ [5–7]. They belong to the superfamily of G-protein-coupled receptors (GPCRs), which contain a conserved structure of seven transmembrane α-helices linked by three alternating intracellular and extracellular loops. According to the classic paradigm of GPCR signaling, binding of the ligand to the receptor induces a sequence of conformational changes that result in its coupling to a heterotrimeric G protein. Activated G proteins then dissociate into G_α and $G_{\beta\gamma}$ subunits, each capable of modulating the activity of a variety of intracellular effector molecules. The protein expression levels of α_1-ARs in mammalian species including humans are considerably lower than for β-ARs [4, 8]. Interestingly, the α_{1A} is predominant α_1-AR in the human heart at the mRNA level, but not at the protein level [9]. Recent evidence suggests

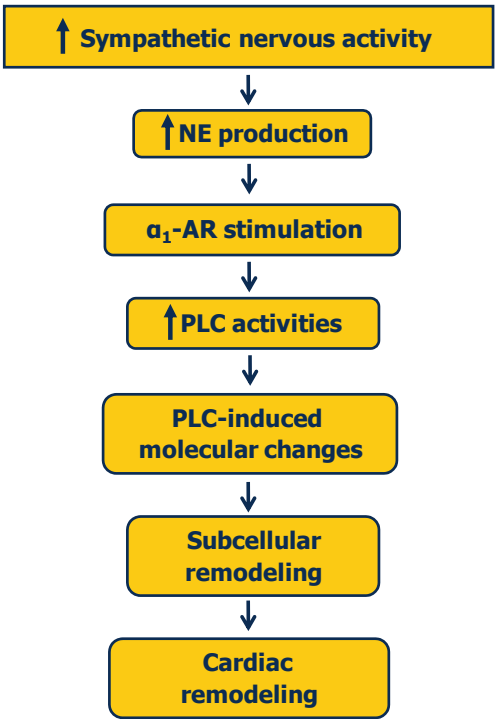

Fig. 17.1 Role of phospholipase C (*PLC*) activation in cardiac remodeling upon stimulation of sympathetic nervous system. *NE* norepinephrine, α_1-*AR* α_1-adrenoceptor

that expression of the α_{1B}-AR may also predominate in the left and right ventricles of the human heart [10].

Both the α_{1A} and α_{1B} subtypes couple to the Gq family of G proteins and are associated with the activation of the cardiac SL membrane-associated phospholipase C β (PLC β) that play a key role in initiation of intracellular signal transduction pathways and regulate a variety of cell functions [11–14]. It is interesting to note that, the proteins involved in targeting PLC β1b to SL membrane have been investigated in neonatal cardiomyocytes. It was found that PLC β1b co-immunoprecipitated with a high-MW scaffolding protein SH3 and ankyrin repeat protein 3 (Shank3) as well as the Shank3-interacting protein α-fodrin, indicating that PLC β1b associates with a Shank3 complex at the SL level [15, 16]. The protein caveolin-3 forms caveolae-flask-shaped invaginations localized on the cytoplasmic surface of the SL membrane [17, 18]. Caveolae have a key role in signal transduction and are gaining more interest as cellular organelles that may contribute to the pathogenesis of cardiac hypertrophy [17, 18]. Interestingly, the α_1-AR, Gq, PLC β_1, and PLC β_3 have been found to be confined exclusively to the same caveolin microdomain in the caveolar fraction isolated from rat heart [19].

The overexpression of α_1-ARs has demonstrated that an increase in α_{1B}-AR, but not α_1-AR activity predisposes the heart to hypertrophy [19]. There is some evidence that the α_{1A}-AR couples to Gq-PLC β more efficiently than the α_{1B}-AR subtype [20]. In this regard, cardiac-specific overexpression of the α_{1A}-AR exerts a

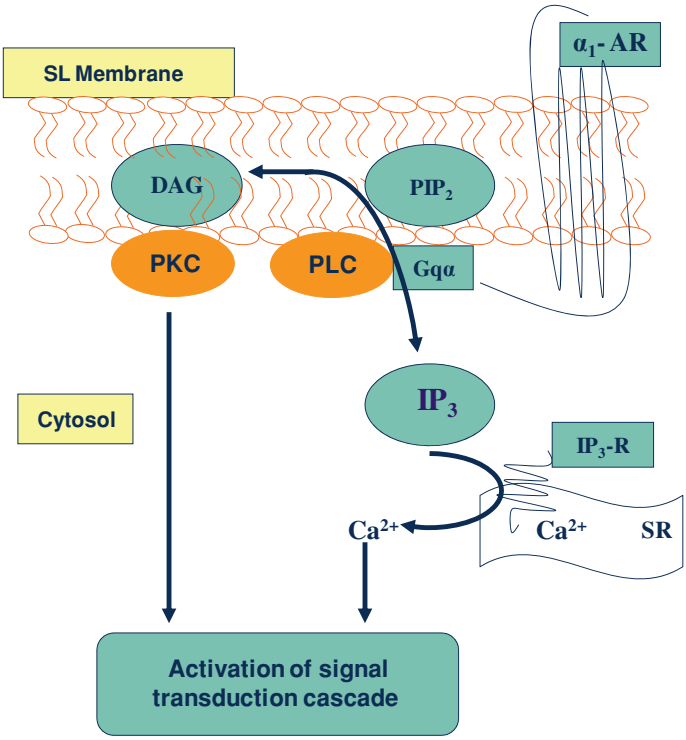

Fig. 17.2 Involvement of different signaling molecules due to the activation of phospholipase C (*PLC*) by α_1-adrenoceptor (α_1-*AR*) for the development of cardiac hypertrophy. *PIP*$_2$ Phosphatidylinositol-4,5-bisphosphate, *DAG* 1,2-diacylglycerol, *IP*$_3$ inositol-1,4,5-trisphosphate, *R* receptor, *Gqα* G-protein qα, *PKC* protein kinase C, *SR* sarcoplasmic reticulum, *Ca*$^{2+}$ calcium ion

higher activation of PLC as compared to α_{1B}-AR overexpression [19, 21]. While PLC β isozymes, β_1 and β_3 have been extensively characterized in cardiac tissue, recently higher PLC β_4 mRNA expression levels than PLC $\beta_{1\text{-}3}$ have been reported in human LV tissue [22]. Furthermore, it was demonstrated that PLC β_4 mRNA levels are increased in response to hypertrophic stimuli in mouse HL-1 cardiomyocytes, suggesting that this isoform may also have a role in the development of cardiac hypertrophy.

17.3 Phospholipase C-Mediated Signal Transduction

The α_1-AR mediated activation of PLC results in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce 1,2 diacylglycerol (DAG), and inositol-1,4,5-trisphosphate (IP_3) (Fig. 17.2). The role of IP_3 in the cardiomyocyte has been a matter for contention as IP_3 generation in cardiomyocytes is low

compared to nonexcitable cells [23, 24]. The IP_3 receptors (IP_3R) are ubiquitous intracellular Ca^{2+} release channels [25]. However, relative to ryanodine receptor (RyR), which is the main source of Ca^{2+} in excitation–contraction coupling (ECC), low levels of IP_3R (approximately 1/50 of RyR) are present in the cardiomyocyte [26, 27]. The type 2 IP_3R, which is the predominant subtype in cardiomyocytes, is located mainly in the nuclear envelope in ventricular cardiomyocytes, but its role in the heart is poorly understood.

It has been suggested that the local Ca^{2+} release results in the activation of transcription, and thus providing a mechanism of how PLC-derived IP_3 may be involved in altered gene expression in cardiac hypertrophy; so-called excitation–transcription coupling [25]. Interestingly, overexpression of IP_3 5-phosphatase has been shown to result in reduced IP_3 responses to α_1-AR agonists acutely, but with prolonged stimulation, an overall increase in PLC activity was observed; this was associated with a selective increase in expression of PLC β_1 that served to normalize IP_3 content in neonatal rat cardiomyocytes [24]. It was suggested that the level of IP_3 selectively regulates the expression of PLC β_1. Furthermore, it was also demonstrated that hearts from type 2 IP_3R knockout mice showed heightened PLCβ_1 expression. Accordingly, it was concluded that IP_3 and type 2 IP_3R regulate PLC β_1 and thereby maintain levels of IP_3 [24], providing further functional significance for IP_3 in the heart. On the other hand, DAG acts in conjunction with phosphatidylserine and in some cases Ca^{2+} to activate different PKC isoforms containing a cysteine-rich C-1 domain that is known to be involved in cardiomyocyte growth [28–31].

17.4 Role of PLC in Different Animal Models of Cardiac Hypertrophy

The role of PLC in the development of different types of cardiac hypertrophy, in vivo, is well documented. For example, the development of cardiac hypertrophy in stroke prone spontaneously hypertensive rats has been reported to involve PLC signaling pathway [32, 33]. In addition, the development of cardiac hypertrophy in cardiomyopathic hamster (BIO 14.6) was found to be associated with an increase in PLC activity [34]. We have previously reported an increase in PLC isozyme gene and protein expression as well as activities in the hypertrophied rat heart; due to volume overload induced by an arteriovenous shunt [35, 36]. Of note, it was demonstrated that increases in PLC β_1 and PLC γ_1 were associated with the hypertrophic stage in this model [36]. Although PLC γ is activated through receptor tyrosine kinase [14, 37], we believe that a reciprocal cross-talk between tyrosine kinase and Gqα may exist in cardiomyocytes [37], linking α_1-AR with tyrosine kinase-associated receptors.

The status of PLC β_1, status in cardiac hypertrophy due to pressure overload induced by ligation of the descending thoracic aorta in the guinea pig, has also been

examined [38]. In this study, quantitative immunoblotting revealed that PLC β_1 and Gαq protein levels were unchanged during hypertrophy. However,translocation of PKC isozymes from cytosol to membranous fractions was elevated. These investigators suggested that PKC translocation occurred without changes in Gαq and PLC β protein abundance and that it might be due to increases in Gαq and PLC β_1 activity rather than upregulation of expression [38]; however, PLC β_1 activity was not determined in this study. Several studies have shown that antagonism of the α_1-AR results in mitigation of cardiac hypertrophy and its progression to heart failure [39–43], thus further implicating PLC β isozymes in the signal transduction mechanisms for cardiac hypertrophy. It should be noted that caveolin-3 expression has been shown to be significantly less in spontaneously hypertensive rats (SHR) as compared to Wistar-Kyoto (WKY) control rats [44]. These investigators suggested that the decrease in caveolin-3 expression may play a role in the development of cardiac hypertrophy in SHR through de-regulating the inhibition of growth signals in the hearts of SHR in the hypertrophic stage. Since α_1-AR and PLC β are located in caveolin-3 [44], it is likely that an increase in α_1-AR-PLC β signal transduction contributes to the cardiac hypertrophy in this model.

17.5 Role of PLC in Cardiac Hypertrophy in Genetic Models

Stimulation of signaling pathways via Gαq and rac1 provokes cardiac hypertrophy in cultured cardiomyocytes and transgenic mouse models [45–48]. The first transgenic murine cardiac hypertrophy model to support a Gαq mechanism of hypertrophy was overexpression of the wild-type Gαq in the heart using the α-MHC promoter [45]. Indeed, a 4-fold overexpression of Gαq resulted in increased heart weight and cardiomyocyte size along with marked increases in atrial natriuretic factor (ANF), α-skeletal actin, and β-myosin heavy chain expression. In view of the fact that an essential downstream effector for Gαq is PLC β [14], these observations would appear to implicate the activation of PLC β isozymes in cardiac hypertrophy. Indeed, Gαq expression in vivo constitutively elevates cardiac PLC β activity [49, 50]. The transgenic mouse line (αq*52) in which cardiac–specific expression of hemagglutinin (HA) epitope-tagged constitutively active mutant of the Gαq subunit (HAαq*) leads to activation of PLCβ, the immediate downstream target of HAαq*, with subsequent development of cardiac hypertrophy and dilation. However, in a second, independent line in the same genetic background (αq*44 h) with lower expression of HAαq* protein that ultimately results in the same phenotype of dilated cardiomyopathy, no correlation with PLC activity was seen [51]. In a different mouse model, loss of PLC ε signaling in PLC ε knockout mice has been suggested to sensitize the heart to the development of hypertrophy in response to chronic isoproterenol treatment [52].

G proteins are subject to direct regulation by RGS (regulators of G protein signaling) proteins, which shorten the duration of the cellular response to external signals and generally cause a reduction in hormone sensitivity [53]. Although the

primary mode of action of RGS proteins is to accelerate termination of the signal by decreasing the lifetime of active, GTP-bound Gα subunits, some RGS proteins can also inhibit signal generation by antagonizing Gα-mediated effector activation [54]. In this regard, recently it has been reported that endogenous ventricular RGS2 expression is selectively reduced in two different models of cardiac hypertrophy (transgenic Gαq expression and pressure overload), which was linked to elevated PLC β activity [55]. These investigators suggested that endogenous RGS2 exerts a functionally important inhibitory restraint on Gq/11-mediated PLC β activation and hypertrophy and concluded that loss of cardiac fine tuning of PLC β signaling by RGS2 down regulation could potentially play a pathophysiological role in the development of Gq/11-mediated cardiac hypertrophy.

The cardiac-targeted overexpression of α_{1A}-AR results in a small increase in the NE-stimulated, but not basal, PLC activity. However, no morphological, histological or echocardiographic evidence of LV hypertrophy was observed [19]. In addition, apart from an increase in ANF mRNA, expression of other hypertrophy-associated genes was unchanged. On the other hand, cardiac-specific expression of α_{1B}-AR in mice results in the activation of PLC as evidenced by an increase in myocardial DAG content [54]. Furthermore, a phenotype consistent with cardiac hypertrophy developed in the adult transgenic mice with increase heart/body weight ratios, cardiomyocyte cross-sectional areas and ventricular ANF mRNA levels [56]. Interestingly, cardiac expression of constitutively active mutant α_{1B}-AR, but not increased expression of α_{1A}-AR has been shown to be involved in the myocardial hypertrophic response to pressure overload in transgenic mice [57, 58]. Thus, it would appear that the α_{1B}-AR is primarily implicated in hypertrophy.

17.6 PLC-Mediated Hypertrophic Responses in Adult Cardiomyocytes

We have earlier reported that the NE-induced increases in ANF (a marker for cardiac hypertrophy), gene expression as well as protein synthesis that can be, in turn, attenuated by U73122, an inhibitor of PLC activities, as well as by an α_1-AR blocker, prazosin in isolated adult rat left ventricular (LV) cardiomyocytes [59]. We have also examined the signal transduction mechanisms involved in the regulation of PLC isozyme gene expression in adult cardiomyocytes in response to NE [60]. In this study, it was revealed that the NE-induced increases in PLC β_1, β_3, γ_1, and δ_1 isozyme mRNA and protein levels were attenuated in cardiomyocytes pretreated with either prazosin, or U73122, an inhibitor of PLC activities. The effects of prazosin and U73122 were associated with inhibition of PLC activity. The inhibition of NE-stimulated PLC protein and gene expression by bisindolylmaleimide-1, a PKC inhibitor, and PD98059, an ERK1/2 inhibitor, indicated that PKC-MAPK may be involved in this signal transduction pathway. Furthermore, significant increases in mRNA levels and protein contents for all PLC isozymes

were found in cardiomyocytes treated with phorbol 12-myristate 13-acetate, a PKC activator. Taken together, it was suggested that PLC isozymes may regulate their own gene expression through a PKC and ERK 1/2-dependent pathway.

An increased expression of the protooncogene, *c-fos* is associated with the initiation of some types of cardiac hypertrophy. In this regard, elevated levels of *c-fos* have been observed in rat heart following administration of NE [61, 62]. Similarly, it has been reported that the stretching of isolated neonatal cardiomyocytes or exposure to NE also elevates *c-fos* mRNA levels and produces cellular hypertrophy [63–65]. Although the pathway that mediates the NE induction of *c-fos* in other cell types has been shown to involve PKC, the identity of the specific PLC isozymes that may be part of this signaling pathway is not known. In addition, since ERK 1/2 is considered to play a major role in the upregulation of the mRNA and protein levels of the immediate early gene *c-jun* [65], it is possible that, this transcription factor may play a role in the regulation of PLC isozyme mRNA levels in response to α_1-AR stimulation in adult cardiomyocytes.

Although it is well-known that both *c-fos* and *c-jun* regulate the expression of a number of genes in the heart [66–69], our studies [70] using *c-fos* and *c-jun* siRNA have indicated that these transcription factors might also regulate the expression of specific PLC isozymes. It should be noted that under our experimental conditions, NE treatment of adult rat cardiomyocytes for 2 h did not induce any change in transcription factors such as NFAT3, NFκB, MEF2C, and MEF2D mRNA levels, suggesting that they may not regulate the early increase in PLC isozyme gene expression in response to NE [71]. Furthermore, our studies revealed that specific PLC isozymes may be involved in the regulation of *c-fos* and *c-jun* gene expression in response to NE [71]. This raises the intriguing possibility of a reciprocal regulation of PLC isozyme and *c-fos*/*c-jun* gene expression in adult cardiomyocytes. In fact, PLC may play an important role in a cycle of events that may be involved in the progression of the cardiomyocyte hypertrophic response (Fig. 17.3).

It should be noted that cardiac hypertrophy independent of PLC activation has also been reported [53, 72]. Nonetheless, from the aforementioned discussion it is possible that specific PLC isozymes might play a contributory role in the signal transduction pathways activated in cardiac hypertrophy. It is worth pointing out that we as well as others have reported that phosphatidic acid (PA), a product of phospholipase D activity, can stimulate PLC isozyme activities [73–75]. We also believe that PA can induce an increase in PLC isozyme gene expression [76]. Interestingly, we have previously reported that PA may be a potential signal transducer for cardiac hypertrophy [73]. In fact, we have also previously reported that PA is a potent stimulator of PLC isozyme activities. Accordingly, it can be suggested that the generation of PA in cardiac hypertrophy may be involved in the perpetuation and amplification of the cardiomyocyte hypertrophic response that might involve increases in PLC isozyme gene and protein expression as well as their activities (Fig. 17.4).

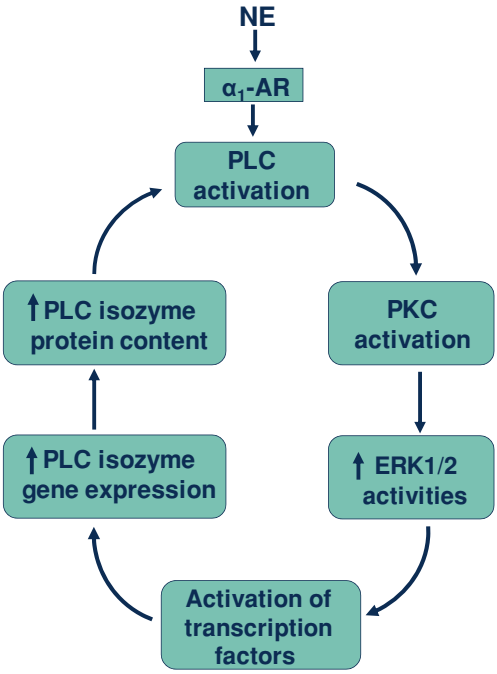

Fig. 17.3 Stimulation of phospholipase C (*PLC*) by norepinephrine (*NE*) mediated cycle of signal transduction events. α_1-*AR* α_1-adrenoceptor, *PKC* protein kinase C, *ERK*1/2 extracellular signal-related kinases 1 and 2

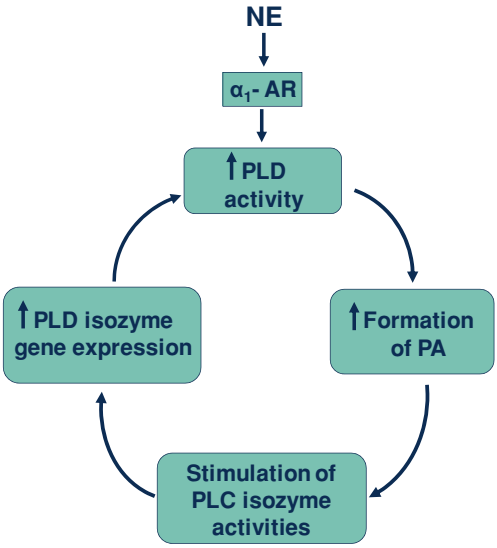

Fig. 17.4 Involvement of phospholipase D (*PLD*) in the activation of phospholipase C (*PLC*) through the formation of phosphatidic acid (*PA*) due to norepinephrine (*NE*). α_1-*AR* α_1-adrenoceptor

17.7 PLC-Mediated Hypertrophic Response in Neonatal Cardiomyocytes

The expression pattern and activation of PLC β isozymes in the development of hypertrophy in neonatal rat cardiomyocytes after stimulation with different hypertrophic substances has been investigated [77, 78]. Under control conditions and after stimulation with NE, cardiomyocytes expressed similar amounts of PLC β_3 mRNA. However, in the presence of fetal calf serum, additional expression of PLC β_1 was induced [77]. The induction of the immediate early genes c-myc, c-fos, and c-jun by IGF-I was also shown to be abolished by preincubation with antisense oligos against PLC β_3. These investigators concluded that the expression of PLC β isozymes in cardiomyocytes is differentially regulated by different hypertrophic stimuli [77]. It is pointed out that the NE-induced IP_3 generation in neonatal rat cardiomyocytes has been reported to be primarily due to α_1-AR mediated activation of PLC β_1 [78]. PLC β_1 exists as two splice variants, PLC β_{1a} and PLC β_{1b}, which differ only in their C-terminal sequences of 64 and 31 amino acids, respectively. While PLC β_{1a} is localized in the cytoplasm, PLC β_{1b} targets to the SL and is enriched in caveolae, where α_1-AR signaling is also localized [79]. Furthermore, in cardiomyocytes, responses initiated by α_1-AR activation involve only PLC β_{1b}, thus the selective action of this splice variant to the SL membrane provides a potential target to reduce hypertrophy [79]. Indeed, recently it has been shown that the overexpression of one splice variant of PLC β_1, specifically PLC β_{1b}, in neonatal rat cardiomyocytes causes increased cell size, elevated protein/DNA ratio, and heightened expression of the hypertrophy-related marker gene, atrial natriuretic peptide [80]. On the other hand, the other splice variant, PLC β_{1a}, had no such effect. Expression of a 32-amino acid C-terminal PLC β_{1b} peptide, which competes with PLC β_{1b} for sarcolemmal association, prevented PLC activation and eliminated hypertrophic responses initiated by Gq or Gq-coupled α_1-adrenergic receptors. In contrast, a PLC β_{1a} C-terminal peptide altered neither PLC activity nor cellular hypertrophy. It was concluded that hypertrophic responses initiated by Gq are mediated specifically by PLC β_{1b}. This study provided further evidence that preventing PLC β_{1b} association with the SL may provide a useful therapeutic target to limit hypertrophy.

PLC ε depletion, using siRNA has been demonstrated to dramatically reduce the hypertrophic growth and gene expression in neonatal rat cardiomyocytes induced by NE, ET-1, IGF-1, and isoproterenol [81]. Furthermore, it was observed that PLC ε catalytic activity was required for hypertrophy development, yet PLC ε depletion did not reduce global agonist-stimulated IP production, suggesting a requirement for localized PLC activity. In fact, these investigators went on to determine that PLC ε is scaffolded to a muscle-specific A kinase anchoring protein (mAKAPβ) that is localized to the nuclear envelope in neonatal rat cardiomyocytes. Accordingly, it was concluded that PLC ε may be involved in the integration of multiple upstream signaling pathways to generate local signals at the nucleus that regulate hypertrophy [81].

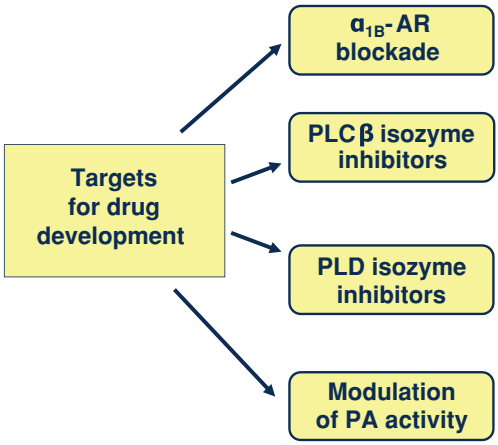

Fig. 17.5 Potential targets in the α_{1B}-adrenoceptor (α_{1B}-*AR*) mediated phospholipase C (*PLC*) signal transduction pathways for the modification of cardiac hypertrophy. *PLD* phospholipase D, *PA* phosphatidic acid

Mechanical stress induced by cell stretching in neonatal cardiomyocytes has also been reported to increase PLC activity [82]. However, in this study no attempt was made to identify the PLC isozymes responsible for such responses. Since mechanical stretch is an initial factor for cardiac hypertrophy in response to hemodynamic overload (high blood pressure) and that increases in Gqα and PLC β_1 activities [38] as well as enhanced NE release from sympathetic nerves [83] are involved in pressure-overload hypertrophy, it is likely that α_1-AR activates PLC β isozymes under conditions of mechanical stress. Indeed, it is important to note that while some studies have reported changes in the expression levels of PLC β isozymes in the hypertrophic response in neonatal cardiomyocytes the signaling function, i.e., PLC activities are determined by the interaction with Gαq, and thus increases in the myocardial PLC isozyme mRNA levels alone, does not necessarily signify a role of PLC isozymes in cardiac hypertrophy.

17.8 Conclusions

The involvement of PLC-mediated signal transduction in cardiac hypertrophy has been demonstrated at the cellular and organ level. While a number of different signal transduction pathways are activated in the myocardial hypertrophic response to different stimuli, it is evident that PLC may constitute additional targets for drug development for the prevention or regression of cardiac hypertrophy in high-risk patients. Although some studies have shown that blockade of the α_1-AR in mitigating the progression of cardiac hypertrophy to heart failure, a direct inhibition of PLC and regression of cardiac hypertrophy is yet to be demonstrated in vivo. Possible targets for drug development for minimizing or reversing cardiac hypertrophy are depicted in Fig. 17.5. The increased formation of PA due to α-AR activation not only stimulate PLC and produce cardiac

hypertrophy, but has also been demonstrated to increase protein synthesis [84]. Interestingly, trimetazidine, an anti-anginal drug has been reported to modulate phospholipid biosynthesis and to reduce IP_3 availability in a PLC-independent manner that results in a prevention of the hypertrophic response to chronic α_1 adrenergic stimulation with phenylephrine in cultured rat cardiomyocytes [85]. The majority of the published work is on describing the involvement of PLCβ isozymes in cardiac hypertrophy; however; since a number of PLC isozymes belonging to different subfamilies (β, γ, δ, and ε) are also expressed in the heart [52, 81, 86, 87] the distinct role of each isozymes, particularly with respect to cardiac hypertrophy, and the extent of their overlap has yet to be completely defined. Indeed, specific PLC isozymes could emerge as important contributors of signal transduction mechanisms for cardiac hypertrophy.

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